U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

## TRANSMITTAL LETTER TO THE UNITED STATES

DESIGNATED/ELECTED OFFICE (DO/EO/US) Us APPLICATION 7 87004 CONCERNING A FILING UNDER 35 USC 371 INTERNATIONAL APPLICATION NO INTERNATIONAL FILING DATE PRIORITY DATE CLAIMED PCT/NL99/00569 14 September 1999 15 September 1998 TITLE OF INVENTION DAIRY PRODUCT AND METHOD FOR PREPARING SAME APPLICANT(S) FOR DO/EO/US Vermin et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information: This is a FIRST submission of items concerning a filing under 35 USC 371. This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 USC 371. 3. A This is an express request to begin national examination procedures (35 USC 371(f)). The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).

 A copy of the International Application as filed (35 USC 371(c)(2)) is attached hereto (required only if not communicated by the International Bureau). As been communicated by the International Bureau.

is not required, as the application was filed in the United States Receiving Office (RO/US).

☐ An English language translation of the International Application as filed (35 USC 371(c)(2)).

7. Amendments to the claims of the International Application under PCT Article 19 (35 USC 371(c)(3)) are attached hereto (required only if not communicated by the International Bureau).

have been communicated by the International Bureau.

have not been made; however, the time limit for making such amendments has NOT expired. have not been made and will not be made.

8. An English language translation of the amendments to the claims under PCT Article 19 (35 USC 371(c)(3)). An oath or declaration of the inventor(s) (35 USC 371(c)(4)).

10. 

An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 USC 371(c)(5)),

11. Nucleotide and/or Amino Acid Sequence Submission

 Computer Readable Form (CRF) Specification Sequence Listing on:

CD-ROM or CD-R (2 copies); or Paper Copy

Statement verifying identity of above copies

Items 12 to 19 below concern other document(s) or information included:

12. An Information Disclosure Statement under 37 CFR 1.97 and 1.98. Form PTO-1449
Copies of Listed Documents

13. 
An assignment for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

14. A FIRST preliminary amendment.

A SECOND or SUBSEQUENT preliminary amendment.

A substitute specification.

16. A change of power of attorney and/or address letter.

17. Application Data Sheet Under 37 CFR 1.76

18. Return Receipt Postcard

19. M Other items or information: Claims as Amended on March 12, 2001

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 U.S. APPLICATION NO
 INTERNATIONAL APPLICATION NO
 ATTORNEY DOCKET NO

 Unassigned
 PCT/NL99/00569
 209684

#### CERTIFICATION UNDER 37 CFR 1.10

"Express Mail" Label Number:

EL643542654US

Date of Deposit:

March 12, 2001

I hereby certify that this express request to begin national examination procedures under 35 USC 371(f) of the International Patent Application referenced above, including all of the items listed thereon as enclosures, is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 CFR 1.10 on the date indicated above and is addressed to Box PCT, Assistant Commissioner for Patents, Attention: DO/EO/US, Washington, D.C. 20231.

Printed Name of Person Signing:

Lasto Owen

JC08 Rec'd PCT/PTO 1 2 MAR 2001

PATENT Attorney Docket No. 209684

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Vermin et al.

Art Unit: Not Assigned

Application No.:

Not Assigned

Examiner: Not Assigned

Filed: March 12, 2001

For: DAIRY PRODUCT AND METHOD FOR

PREPARING SAME

#### PRELIMINARY AMENDMENT

Commissioner for Patents Washington, D.C. 20231

Dear Sir:

Prior to the examination of the above-identified patent application, please enter the following amendments and consider the following remarks.

### AMENDMENTS

#### IN THE SPECIFICATION:

Page 1, below the title, please insert the following paragraph:

## CROSS REFERENCE TO RELATED APPLICATION

The present application claims priority of Vermin et al., PCT

Application PCT/NL99/00569 filed on September 14, 1999, and Vermin et al., Netherlands Application Serial No. 1010096 filed on September 15, 1998 entitled "Dairy Product and Method for Preparing Same."

Page 1, line 1, please insert the following heading:

AREA OF THE INVENTION

Page 1, line 4, please insert the following heading:

BACKGROUND OF THE INVENTION

Page 2, line 20, please insert the following heading:

SUMMARY OF THE INVENTION

Page 2, line 34, please insert the following heading:

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

## IN THE CLAIMS:

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Please amend claims 1-9 to read as follows:

(Amended) A method for preparing a dairy product comprising the steps of: treating a medium comprising milk or a milk product under aerobic conditions with a lactose-negative, food-technologically acceptable microorganism to render a treated medium comprising milk or a milk product, and

maintaining the treated medium comprising milk or a milk product under anaerobic conditions

2. (Amended) The method according to claim 1, in which the microorganism is a yeast.

- (Amended) The method according to claim 2, in which the yeast originates from one of the following set of strains: Candida zelanoides, Debaryomyces hansenii spp hansenii, Saccharomyces cerevisiae, Candida robusta, or Zygosaccharomyces rouxii.
- (Amended) The method according to claim 10, in which the bacterium originates from one of the following set of strains: Micrococcus luteus, Arthrobacter, Corynebacterium or Arthrobacter ssp.
- (Amended) The method according to claim 1, in which the medium is treated under anaerobic conditions with a food-technologically acceptable lactic acid bacterium.
- (Amended) The method according to claim 1 in which the dairy product is thermized.
- 7. (Amended) A dairy product prepared by treating a medium comprising milk or a milk product under aerobic conditions with a lactose-negative, food-technologically acceptable microorganism to render a treated medium comprising milk or a milk product; and

maintaining the treated medium comprising milk or a milk product under anaerobic conditions.

8. (Amended) A method for producing a food comprising a dairy product comprising: introducing a lactose-negative, food-technologically acceptable microorganism to a medium comprising milk or a milk product, thereby imparting an aroma to the dairy product.  (Amended) The method according to claim 7 further comprising preparing an aromatized food product including the dairy product.

Please add the following new claims 10-20:

- (New) The method according to claim 1, in which the microorganism is a bacterium.
  - 11. (New) The method of claim 2 wherein the dairy product is thermized.
  - 12. (New) The method of claim 3 wherein the dairy product is thermized.
  - 13. (New) The method of claim 3 wherein the dairy product is thermized.
  - 14. (New) The method of claim 3 wherein the dairy product is thermized.
- (New) The dairy product according to claim 7, in which the microorganism is a yeast.
- 16. (New) The dairy product according to claim 15, in which the yeast originates from one of the following set of strains: Candida zelanoides, Debaryomyces hansenii spp hansenii, Saccharomyces cerevisiae, Candida robusta, or Zygosaccharomyces rouxii.
- (New) The dairy product according to claim 7, in which the microorganism is a bacterium.
- 18. (New) The method according to claim 17, in which the bacterium originates from one of the following set of strains: Micrococcus luteus, Arthrobacter, Corynebacterium or Arthrobacter ssp.
- (New) The method according to claim 7, in which the medium is treated under anaerobic conditions with a food-technologically acceptable lactic acid bacterium.

 (New) The method according to claim 7 in which the dairy product is thermized.

#### REMARKS

The application is considered in good and proper form for allowance, and the Examiner is respectfully requested to pass this application to issue. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

Mark Joy, Reg. No. 35,562

One of the Attorneys for Applicant(s) LEYDIG, VOIT & MAYER, LTD.

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Date: March 12, 2001

JC08 Rec'd PCT/PTO

1 2 MAR 2001

PATENT Attorney Docket No. 209684

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Vermin et al.

Not Assigned

Art Unit: Not Assigned

Examiner: Not Assigned

For: DAIRY PRODUCT AND METHOD FOR

PREPARING SAME

#### CLAIMS AS AMENDED ON MARCH 12, 2001

1. (Amended) A method for preparing a dairy product [, in which] <u>comprising the steps of:</u>

treating a medium comprising milk or a milk product [is treated] under aerobic conditions with a lactose-negative, food-technologically acceptable microorganism to render a treated medium comprising milk or a milk product, and

maintaining the treated medium comprising milk or a milk product [is then kept] under anaerobic conditions [for some time].

- 2. (Amended) [A]  $\underline{\text{The}}$  method according to claim 1, in which the microorganism  $\underline{\text{is}}$  a yeast [or a bacterium is].
- 3. (Amended) [A] The method according to claim 2, in which the yeast originates from one of the following set of strains: [strain] Candida zelanoides, Debaryomyces hansenii spp hansenii, Saccharomyces cerevisiae, Candida robusta, or Zygosaccharomyces rouxii.

- (Amended) [A] The method according to claim [2] 10, in which the bacterium originates from one of the following set of strains: [strain] Micrococcus luteus, Arthrobacter, Corynebacterium or Arthrobacter ssp.
- (Amended) [A] <u>The</u> method according to claim 1, in which the medium is treated under anaerobic conditions with a food-technologically acceptable lactic acid bacterium.
- (Amended) [A] <u>The</u> method according to <u>claim 1</u> [any of the preceding claims] in which the dairy product is thermized.
- 7. (Amended) A dairy product [obtainable] <u>prepared</u> by [a method according to any of the preceding claims] <u>treating a medium comprising milk or a milk product under aerobic conditions with a lactose-negative, food-technologically acceptable microorganism to render a <u>treated medium comprising milk</u> or a milk product; and</u>

maintaining the treated medium comprising milk or a milk product under anaerobic conditions.

8. (Amended) A method for producing a food comprising a dairy product comprising:

[The use of] introducing a lactose-negative, food-technologically acceptable microorganism [for] to a medium comprising milk or a milk product, thereby imparting an aroma to [a] the dairy product.

- (Amended) The [use of a dairy product] method according to claim 7 <u>further</u>
   <u>comprising preparing</u> [for the preparation of] an aromatized <u>food</u> product [in the food industry]
   <u>including the dairy product</u>.
- (New) The method according to claim 1, in which the microorganism is a bacterium.
  - 11. (New) The method of claim 2 wherein the dairy product is thermized.
  - 12. (New) The method of claim 3 wherein the dairy product is thermized.
  - 13. (New) The method of claim 3 wherein the dairy product is thermized.
  - 14. (New) The method of claim 3 wherein the dairy product is thermized.
- (New) The dairy product according to claim 7, in which the microorganism is a yeast.
- 16. (New) The dairy product according to claim 15, in which the yeast originates from one of the following set of strains: Candida zelanoides, Debaryomyces hansenii spp hansenii, Saccharomyces cerevisiae, Candida robusta, or Zygosaccharomyces rouxii.
- (New) The dairy product according to claim 7, in which the microorganism is a bacterium.

- 18. (New) The method according to claim 17, in which the bacterium originates from one of the following set of strains: Micrococcus luteus, Arthrobacter, Corynebacterium or Arthrobacter ssp.
- 19. (New) The method according to claim 7, in which the medium is treated under anaerobic conditions with a food-technologically acceptable lactic acid bacterium.
  - 20. (New) The method according to claim 7 in which the dairy product is thermized.

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Title: Dairy product and method for preparing same.

The invention relates to a method for preparing a dairy product and to a dairy product obtainable by this process.

In the course of years more and more different types of dairy products have been developed. The consumer can choose among a wide range of products, such as custard in all kinds of flavors, ice cream, yogurt and yogurt beverages, quark (curd cheese), Biogarde®, sour cream, whipped cream and crème fraîche.

A number of the available dairy products are so-called fermented dairy products. That is to say that in the preparation of these products a fermentation step is carried out. In such a fermentation step the product is treated with microorganisms and/or enzyme systems which converts the lactose present in the product to a greater or lesser extent. Such fermentations yield, depending on the employed microorganism or mixtures of microorganisms and enzymes, a dairy product having a characteristic flavor and texture. Examples of fermented dairy products are yogurt, Biogarde<sup>6</sup>, Biomild<sup>6</sup>, quark and sour cream.

In order to give dairy products, both fermented and non-fermented, a specific desired flavor, diverse additives are added. Many employed additives are sweeteners, flavorings, aromatics and colorings. In products such as custard, fruit yogurt and yogurt beverages, it is conventional to use artificial additives, fruit juices or plant extracts.

Of a number of dairy products the consumer wishes a natural variant. By this is meant a dairy product which owes its characteristic appearance and flavor to the nature of the dairy product itself, without additives being added. Dairy products of which in particular the availability of natural variants is expected are yogurt, quark, buttermilk and the like.

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U.S. patent 4,675,193 discloses a two-step method for preparing a product having a cheese flavor. To this end, in the first step a flavor developing medium having a fat content between 0.5 and 50% (w/v) and a protein content between 0.3 and 12% (w/v), for instance milk or whey, is treated with a lipase and/or protease source. This step is carried out under aerobic conditions, after which the employed lipase and/or protease source is deactivated. In a second step a fermentation with a lactic acid producing microorganism is carried out.

As the lipase and/or protease source, non-pathogenic microorganisms are preferably used, such as Penicillium roqueforti, Oidum lactis (Geotrichum candidum), Cladisporum butaryl, Micrococcus and Candida lipsolytica. Preferably, C. lipsolytica is used.

The treatment step in which the flavor developing medium is used comprises a fermentation in which the lipase and/or protease source acidifies the medium to a pH between 4.5 and 5.5, followed by a deactivation.

It is an object of the invention to provide a dairy product which as regards texture corresponds to a known dairy product, such as yogurt or quark, but which has a different flavor and/or odor.

Surprisingly, it has been found that such dairy products can be prepared by bringing a specific aroma into the dairy product, which aroma is produced by a specific aerobic microorganism which is kept under anaerobic conditions for some time. Preferably, a conventional fermentation is carried out during this anaerobic period.

The invention relates to a method for preparing a dairy product in which a medium comprising milk or a milk product is treated under aerobic conditions with a lactosenegative, food-technologically acceptable microorganism.

It has been found that in a medium treated under aerobic conditions with a lactose-negative, food-technologically acceptable microorganism during an

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anaerobic period there are formed characteristic aromas. The nature of the aroma can be adjusted by the choice of the microorganism within the class of lactose-negative, food-technologically acceptable microorganisms.

The aroma imparted according to the invention to a dairy product originates from a microorganism which hardly changes the starting material for the dairy product to be prepared in the sense that lactose is not converted. There is thus obtained a dairy product which as regards the texture is substantially equal to known variants of the dairy product prepared, but which has a completely new flavor and/or odor.

On the other hand, the use of a lactose-negative microorganism in the preparation of a dairy product is known per se. In J. Dairy Sci., (1996) 79:937-942, Kwak et al. describe a method in which a lactose-negative yeast of the species Saccharomyces cerevisiae is used to stabilize kefir. Kefir is a traditional, Russian, fermented milk beverage containing a minor amount of alcohol and carbon dioxide. According to Kwak et al. the lactose-negative microorganism is used to produce alcohol in a preliminary phase under anaerobic conditions. After this preliminary phase an acidification with lactic acid bacteria is carried out.

The starting material for the method according to the invention is a medium comprising milk or a milk product. It is possible to use both a non-treated milk and a milk which has been subjected previously to a heat treatment, such as a pasteurization or sterilization.

In order to prevent outgrowth of any present, contaminating microorganisms, it is preferred to start from a milk which has been previously subjected to a heat treatment.

It is also possible to use a milk-derived product
having an adapted fat content, such as skim milk or cream.

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In addition to the milk or the milk product, the medium can and will generally comprise nutrients for the lactose-negative, food-technologically acceptable m.croorganism. It is conventional to subdivide these ru rients into carbon sources and nitrogen sources. (u.table carbon sources are substances which can supply the carbon required for the ripening of the lactose-negative, fcod-technologically acceptable microorganism, and which are compatible with the dairy product (to be prepared). Examples are glucose and potato dextrose broth (PDB). Euitable nitrogen sources are substances which can supply the nitrogen required for the ripening of the lactosenegative, food-technologically acceptable microorganism and have no negative effect on the dairy product (to be prepared). Examples are malt extract, yeast extract and tryptonē.

The choice of the nutrients to be used will depend on the nature of the lactose-negative, food-technologically acceptable microorganism. When the lactose-negative, food-technologically acceptable microorganism is a yeast, glucose, PDB and tryptone are preferably used as nutrients. When the lactose-negative, food-technologically acceptable microorganism is a bacterium, glucose and/or tryptone are preferably used as nutrients.

The amounts of the nutrients in the medium which form the starting material for a method according to the invention are adjusted to the nature of the lactosenegative, food-technologically acceptable microorganism. The total amount of added nutrients required for the aromaforming microorganism should be sufficient to grow the microorganism, but not so large as to bring about an undesired aroma or an aroma of an undesired intensity. When the carbon source is PDB, in the case that the lactosenegative, food-technologically acceptable microorganism is a yeast, the amount thereof will range between 1 and 3 g. When in the same case tryptone is the nitrogen source, 0.2

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to 1 g thereof will be used. When the carbon source is glucose, in the case that the lactose-negative, food-technologically acceptable microorganism is a bacterium, the amount thereof will range between 0.5 and 5 g. When in the same case tryptone is the nitrogen source, 0.2 to 1 g thereof will be used. All this can be simply established by those skilled in the art by way of experiment.

The microorganism with which the medium according to the invention is treated is a lactose-negative, food-technologically acceptable microorganism. By this is meant a microorganism which is not capable of converting lactose. In other words, when only lactose is present as the carbon source, the microorganism cannot grow.

Preferably, the lactose-negative, food-technologically acceptable microorganism is a yeast or a bacterium. Very suitable yeast strains are Candida zelanoides CBS 2328, Candida zelanoides ATCC 26318, Debaryomyces hansenii CBS 772, Debaryomyces hansenii ATCC 10623, Saccharomyces cerevisiae CBS 400, Saccharomyces cerevisiae CBS 437, Candida robusta CBS 420 and Zygosaccharomyces rouxii CBS 441. Very suitable bacteria originate from the strains Micrococcus luteus ATCC 4698, Micrococcus luteus CIP A270, Arthrobacter nicotianea ATCC 21279 and Corynebacterium flavescens ATCC 10340.

25 It is also possible to use combinations of lactosenegative, food-technologically acceptable microorganisms.

The choice of the lactose-negative, food-technologically acceptable microorganism or the combination of lactose-negative, food-technologically acceptable microorganisms can be suitably adjusted to the desired aroma. It has been found that by means of the treatment with the above microorganisms there can be produced a wide range of aromas, including caramel, chocolate, cheesy, nutty, garlic, and a neutral aroma which, however, makes the flavor of the fermentation product richer and rounder.

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In order to obtain the desired aroma, the medium comprising the milk or the milk product is treated with the lactose-negative, food-technologically acceptable microorganism. This lactose-negative, food-technologically acceptable microorganism is added to the medium and ripened. The amount of the lactose-negative, food-technologically acceptable microorganism can be suitably adjusted to the amount of medium and the amount (the strength) of the desired aroma. The lactose-negative, food-technologically acceptable microorganism will generally be used in an amount of 106 to 107 per ml of milk or milk product.

The ripening of the lactose-negative, food-technologically acceptable microorganism is effected under aerobic conditions. The pH and the temperature during the ripening will depend on the employed specific lactose-negative, food-technologically acceptable microorganism. In most cases the pH will range between 6 and 7. The temperature will mostly be chosen between 25 and 30°C.

Preferably, the ripening is effected so as to be complete. The duration of the ripening depends on the stationary growth phase of the employed microorganism, and will generally range between 12 and 24 hours. This has the result that the nutrients present in the medium are consumed and remainders of any nutrients added will hardly, if at all, find their way into the ready dairy product. The moment at which the ripening is complete, can be suitably determined by following the content of one or more of the nutrients, for instance of glucose. It is also possible to follow the growth of the lactose-negative, food-technologically acceptable microorganism by means of conventional plate counts.

After the ripening the resulting product is brought under anaerobic conditions for some time. It has been found that in the absence of oxygen the aromas of the ripening strain are formed and are excellently expressed.

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During the aroma formation a neutral pH is preferably used. In most cases a period of 5 to 12 hours will be sufficient to obtain a proper aroma formation.

The thus obtained product is suitable per se for consumption. It will be clear that the formation of the desired aromas can take place for instance after the dairy product has been packaged into a container in which conventional anaerobic conditions prevail. Often, however, the resulting product will be thermized to increase the keeping quality. Furthermore, depending on the desired nature of the dairy product prepared, additional operations can be carried out.

In a preferred embodiment the anaerobic conditions coincide with a fermentation step. After addition of conventional fermentation strains an anaerobic fermentation is carried out in which lactose is converted into, inter alia, lactic acid.

More in detail, the product obtained after the ripening of the lactose-negative, food-technologically acceptable microorganism is treated with a lactic acid bacterium. Because of this treatment, the dairy product, particularly as regards texture and acidity, will showgreat similarities to known fermented dairy products, such as yogurt or quark. On account of the change in the acidity in a treatment with a lactic acid bacterium, this treatment is often designated as acidification.

The nature of the lactic acid bacterium will be suitably adjustable to the desired product by those skilled in the art. The treatment with the lactic acid bacterium can be carried out in the known manner and will be analogous to the treatment of a milk or milk product with a lactic acid bacterium in the preparation of fermented dairy products which have not been treated with a lactosenegative, food-technologically acceptable microorganism.

It will be clear that the invention also relates to a dairy product that can be prepared by a method as

described above. Furthermore, the invention relates to the use of a dairy product that can be prepared according to the invention in the preparation of automated products in the food industry.

The invention will now be further explained on the basis of the following examples.

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#### Example 1

#### Yeast strairs

Candid: zelanoides CBS 2328 from ampoule
Candida zelanoides ATCC 26318 from ampoule
Debaryomyces hansenii ATCC 10623 from ampoule
Debaryomyces hansenii ATCC 772 from ampoule

#### Bacterial strain:

Arthrobacter nicotianea ATCC 21279 from plate

#### Glucose sc .t tion:

8 g cf glucose per 100 g of water (filter sterilized)
Yeast med grad

24 g cī potato dextrose broth (PDB of Difco 0549-17-9)

+ 5 % of tryptone per liter of water.

## Bacterial medium:

5 g of tryptone per 900 ml of water. Sterilized at 120 C for 15 min and enriched with 100 ml of glucose solit on

### Substrat 2.

whole UHT-milk

#### Cu ture

A sclony of lactose-negative microorganisms is added to water with glycerol and the solution is distributed over several ampoules and frozen. Subsequently, 1 ml of thawed medium is added to 25 ml of yeast of bacterial medium in an Erlenmeyer of 100 ml. The Erlenmeyer is closed with cellulose wadding and aerobically stored for 72 hours at  $25\,^{\circ}\mathrm{C}$  (yeasts) and 30°C (bacteria) at a shaking speed of 200 rpm.

#### Pra-culture

5 ml of culture medium is added to 45 ml of whole UHT milk (substrate) in an Erlenmeyer of 250 ml which is closable with wadding. This solution is aerobically stored for 14 hours or 12 hours with shaking at 200 rpm and at the right temperature. After the pre-culture has been finished, the media are cooled to ca. 7°C.

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#### Result

After an aerobic growth of the lactose-negative, food-technologically acceptable yeast Candida zeylanoides and an anaerobic aroma formation in milk, there is formed a product with a distinguishing flavor, to be associated with Munster cheese (stable flavor). When the starting material is lactose-negative bacterium Arthrobacter, a chocolate flavor is formed after an anaerobic aroma formation in milk. In this product branched chain aldehydes were the most important compounds that could be detected with "dynamic headspace GCMS".

## Example 2

#### Yeast strains:

Candida zelanoides CBS 2328 from ampoule Candida zelanoides ATCC 26318 from ampoule

#### Bacterial strain:

Arthrobacter nicotianea ATCC 21279 from plate Lactic acid bacteria:

20 For the preparation of yogurt

#### Glucose solution:

8 g of glucose per 100 g of water (filter sterilized) Yeast medium:

24 g of potato dextrose broth (PDB of Difco 0549-17-9)

+ 5 g of tryptone per 1 of water.

#### Bacterial medium:

5 g of tryptone per 900 ml of water. Sterilized at  $120\,^{\circ}\text{C}$  for 15 min and enriched with 100 ml of glucose solution

#### 30 Substrate:

whole UHT-milk

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#### Culture

A colony of lactose-negative microorganism is added to water with glycerol and the solution is further distributed over several ampoules and frozen. Subsequently, 1 ml of thawed medium is added to 25 ml of yeast or bacterial medium in an Erlenmeyer of 100 ml. The Erlenmeyer is closed with cellulose wadding and aerobically stored for 72 hours at 25°C (yeasts) and 30°C (bacteria) at a shaking speed of 200 rpm.

#### 10 Pre-culture

5 ml of culture medium is added to 45 ml of whole UHT milk (substrate) in an Erlenmeyer of 250 ml which is closable with wadding. This solution is aerobically stored for 24 hours or 12 hours with shaking at 200 rpm and at the right temperature. After the pre-culture has been finished, the media are cooled to ca. 7°C.

#### Acidification

The pre-culture is acidified under anaerobic conditions with a yogurt culture at  $37^{\circ}\text{C}$  to final pH 4.5 and further packaged in cooled condition (to ca. 5-7°C).

#### Result

When both products obtained in Example I are acidified with lactic acid bacteria, there are formed products having a consistency comparable to that of yogurt and having a distinguishing flavor clearly different from the flavor of a non-pretreated, acidified milk product.

#### CLAIMS

- 1. A method for preparing a dairy product, in which a medium comprising milk or a milk product is treated under aerobic conditions with a lactose-negative, food-technologically acceptable microorganism, and is then kept under anaerobic conditions for some time.
- 2. A method according to claim 1, in which the microorganism a yeast or a bacterium is.
- 3. A method according to claim 2, in which the yeast originates from the strain Candida zelanoides, Debaryomyces hansenii spp hansenii, Saccharomyces cerevisiae, Candida robusta, or Zygosaccharomyces rouxii.
- 4. A method according to claim 2, in which the bacterium originates from the strain Micrococcus luteus, Arthrobacter, Corynebacterium or Arthrobacter ssp.
- 15 5. A method according to claim 1, in which the medium is treated under anaerobic conditions with a foodtechnologically acceptable lactic acid bacterium.
  - 6. A method according to any of the preceding claims, in which the dairy product is thermized.
- 20 7. A dairy product obtainable by a method according to any of the preceding claims.
  - 8. The use of a lactose-negative, food-technologically acceptable microorganism for imparting an aroma to a dairy product.
- 25 9. The use of a dairy product according to claim 7 for the preparation of an aromatized product in the food industry.

## SU'G PCETTIO 23 MAY 2001 **Declaration and Power of Attorney Patent Application**

# (Design or Utility)

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: 'Dairy product and method for preparing same'

the specification of which is attached hereto

	is attached in		
X	was filed on	March 12, 20	as application serial no. 09/787,004
	and or PCT In	nternational App	olication number PCT/NL99/00569 and was amended
	on		applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information know to me to be material to patentability as defined in 37 C.F.R.§1.56.

I hereby claim foreign priority benefits under 35 U.S.C.§119(a)-(d) or 35 U.S.C.§365(b) of any foreign application(s) for patent or inventor's certificate, or 35 U.S.C.§365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate of PCT International application having a filing date before that of the application on which priority is claimed.

	Prior Foreign App	lication(s)
Number 1010096	Country NL	Day/Month/Year Filed 15-09-1998
Number	Country	Day/Month/Year Filed
Number	Country	Day/Month/Year Filed

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

Prior Provisional Application(s)		
Serial Number	Day/Month/Year Filing Date	
Serial Number	Day/Month/Year Filing Date	
Serial Number	Day/Month/Year Filing Date	

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s), or under 35 U.S.C. §365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R.§1.56 which became available between the filling date of the prior application and the national or PCT International filing date of this application:

Prior U.S. or International Application(s)			
Serial Number	Day/Month/Year Filed	Status (patented, pending, abandoned)	
Serial Number	Day/Month/Year Filed	Status (patented, pending, abandoned)	
Serial Number	Day/Month/Year Filed	Status (patented, pending, abandoned)	

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C.§1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

## Power of Attorney

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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